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FLUORESCENT TREPONEMAL ANTIBODY TEST*

A PRELIMINARY REPORT

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The fluorescent treponemal antibody (F.T.A.) test described by Deacon, Falcone, and Harris (1957) was one of the procedures using treponemal antigens chosen for inclusion in the Serology Evaluation and Research Assembly (S.E.R.A.) Study carried out under the auspices of the United States Public Health Service in 1956–57 (U.S.P.H.S. Report, 1959). The test was shown to give very sensitive and specific results and compared favourably with the other treponemal tests used. Other workers (Censuales and Garofalo, 1959; Borel and Durel, 1959; Thivolet, Grospiron, and Murat, 1960) have also reported their findings on small numbers of sera. The present communication describes the results obtained in tests on sera from presumed normal individuals, patients with treponemal infection, and patients whose sera were suspected to have given non-specific reactions with lipoidal antigen tests.

Technique

The technique used closely followed the modified method described by Deacon, Freeman, and Harris (1960).

Anti-Human Globulin Serum.—This was prepared by injecting 5 mg. human gamma globulin with Freund's adjuvant into the hind leg muscles of rabbits. A booster dose of 5 mg. human gamma globulin without adjuvant was given about 3 months later and the animals were bled out after an interval of 10 to 14 days when a satisfactory level of antibody was demonstrable by a precipitin (ring) test. The globulin fraction of the serum was separated by precipitation with ammonium sulphate and conjugated with fluorescein isothiocyanate by the method of Marshall, Eveland, and Smith (1958). The conjugate was titrated by an optimal proportions procedure in the F.T.A. test against a known positive serum, and was used at the highest dilution which gave a definite reactive reading with the greatest dilution of the control serum.

Treponeme Suspension.—This was prepared in *Treponema pallidum* immobilization (T.P.I.) test medium from rabbits treated with cortisone and infected intratesticularly with the Nichols strain of *T. pallidum*. It was adjusted to a density of 50 to 75 organisms per high dry field and 1 mg./ml. streptomycin was added to inhibit the growth of bacterial contaminants. Suspensions so prepared remained stable in the refrigerator for several months, but before being taken into use were checked to ensure that they did not show non-specific staining with the conjugate.

Sera.—These were stored at −20°C. and inactivated at 56°C. for 30 minutes before testing. If re-tested they were again heated at 56°C. for 10 minutes.

Buffered Saline.—pH 7·2–7·3, NaCl 6·8 g., Na_2HPO_4 1·48g., KH_2PO_4 0·43 g. in one litre distilled water.

Technique of Test

(1) Circles 1 cm. in diameter were inscribed on microscope slides with a diamond and a 4 mm. loopful of treponeme suspension was spread within the marked area and allowed to dry in the air. The slides were then fixed in acetone for 10 to 15 minutes.

(2) 0·03 ml. of a 1 in 200 dilution of serum in buffered saline was added to the fixed treponemes with a dropping pipette, and the slides were incubated in a moist chamber for one hour at 37°C.

(3) Excess serum was washed off gently with buffered

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saline and the slides soaked in two changes of buffered saline for 5 minutes.

(4) After removing excess buffer, 0·03 ml. conjugate diluted to its titre in 2 per cent. Tween 80 in buffered saline was added, and the slides were incubated for a further 30 minutes at 37°C. in a moist chamber.

(5) After excess conjugate had been washed off, the slides were soaked for 5 minutes in two changes of buffered saline, and mounted in a drop of a mixture of nine parts of glycerine and one part buffered saline.

Optical Equipment.—The ultraviolet source used was a 250 watt ME/D high-pressure mercury vapour lamp with a condensing lens. The light was passed through a 1 cm. trough of 5 per cent. copper sulphate and a Chance-Watson OX 7 filter transmitting in the 2500–3900 Å region. A Beck focusing dark-ground condenser was used with a Zeiss E objective and a X4 eyepiece fitted with a Chance OY 12 filter to prevent any residual ultraviolet light from reaching the eye. The microscope and illuminating system were fixed on a baseboard and the observations were made in a darkroom.

Reading of Results.—Judgment of the intensity of fluorescence is of necessity subjective, but the following scale was adopted:

(*a*) ++++ Very brilliant apple-green fluorescence.
(*b*) +++ Brilliant fluorescence
(*c*) ++ Definite fluorescence, not so marked as in (*b*)
(*d*) + Faint fluorescence
(*e*) ± Treponemes just visible, but no green fluorescence
(*f*) 0 Treponemes not visible

Grades (*a*), (*b*), and (*c*) were classed as "Reactive" and grades (*d*), (*e*), and (*f*) as "Non-reactive". When no treponemes could be seen the slide was checked by dark-ground examination with visible light to ensure that the organisms had not been accidentally removed from the slide during the staining procedures. Strongly reactive, weakly reactive, and negative sera were included as controls in each batch of tests.

Other Serological Tests

Wassermann Reaction (W.R.) using crude heart extract and Maltaner cardiolipin antigens in the Whitechapel W.R. technique (Price, 1949; 1950a, b).

Price's Precipitation Reaction (P.P.R.). (Price, 1948).

Reiter Protein Complement-Fixation Test (R.P.C.F.T.) using antigen obtained from Organon Laboratories and used in the Whitechapel Wassermann technique at a titre of 1 in 80.

Treponemal Immobilization (T.P.I.) *Test.*—18 hrs incubation at 35°C. using 30 per cent. complement in the reaction mixture.

Results

(A) Presumed Non-syphilitic Group.—These sera were obtained from 163 antenatal patients, whose W.R. with crude heart extract antigen and P.P.R. had been found negative on routine testing, and from four members of the laboratory staff whose S.T.S. and T.P.I. tests were known to be negative. No reactive F.T.A. tests were found in this group of 167 sera.

(B) Patients with Treponemal Infection.—A comparison of the results of tests on the same specimens of sera by the F.T.A. and other tests is shown in Tables I to III. For simplicity the results of the three tests using lipoidal antigens (W.R. with crude heart extract and with cardiolipin antigens and the P.P.R.) have been grouped together as "S.T.S.". Positive and weakly positive results have been classed as "Reactive" (R), and negative results as "Non-reactive" (N.R.).

In the small number of sera available from patients with early syphilis (Table I), the F.T.A. was found to be at least as sensitive as the other two types of test. It was reactive with sera from two patients with darkfield positive primary lesions when both the R.P.C.F.T. and S.T.S. gave negative results. In the treated group of patients antibody was also detectable by the fluorescence procedure when the other tests were negative.

TABLE I

COMPARISON OF RESULTS OF TESTS ON SERA FROM PATIENTS WITH EARLY SYPHILIS

Test	Result	Cases of Early Syphilis	
		Untreated Primary 7 Secondary 5	Treated Primary 10 Secondary 5
F.T.A.	R	11	6
	N.R.	1	9
R.P.C.F.T. ..	R	10	4
	N.R.	2	11
S.T.S.	R	10	3
	N.R.	2	12
Total ..		12	15

The majority of the patients in whom a diagnosis of latent treponemal disease had been made were coloured, most of them coming from the West Indies. A history of yaws in early life was frequently given; as this was often based only on a recollection of having had injections as a child, the accuracy of the diagnosis is questionable. It has therefore been thought preferable to class these patients under the general heading of latent treponemal disease. Patients were included in this group only if the T.P.I. test was positive (on earlier specimens of serum in the treated group), if they had latent congenital infections, or if their sexual contacts were found to be sero-positive. The results are summarized in Table II.

TABLE II

COMPARISON OF RESULTS OF TESTS ON SERA FROM PATIENTS WITH LATENT TREPONEMAL INFECTION

Test	Result	Cases of Latent Infection	
		Untreated	Treated
F.T.A.	R	34	52
	N.R.	1	5
R.P.C.F.T. ..	R	27	31
	N.R.	8	26
S.T.S.	R	31	42
	N.R.	4	15
Total		35	57

The F.T.A. test was found to be more sensitive than the other procedures, particularly in patients who had been treated in the past. Only one serum in the untreated group was classed as non-reactive, and this showed weak (+) fluorescence. The result of tests on sera from 41 patients with late symptomatic syphilis are shown in Table III. The treated group includes eight patients who had late manifestations of congenital infection.

TABLE III

COMPARISON OF RESULTS OF TESTS ON SERA FROM PATIENTS WITH LATE SYMPTOMATIC SYPHILIS

Test	Result	Cases of Late Syphilis	
		Untreated	Treated
F.T.A.	R	10	25
	N.R.	0	6
R.P.C.F.T. ..	R	9	17
	N.R.	1	14
S.T.S.	R	10	23
	N.R.	0	8
Total		10	31

The F.T.A. test showed a high level of reactivity in this group of sera. Three of the six patients whose F.T.A. tests were negative were congenital syphilitics who had suffered from interstitial keratitis in the past, and a further patient had had tabes 15 years previously. It was noted that T.P.I. tests performed on sera from three of these patients showed only a low titre (33, 63, and 85 per cent. specific immobilization). A further patient had been treated for a gummatous lesion of the mouth 32 years before. The last case in this group was a West Indian with bone lesions who had been treated 9 months before the F.T.A. test was performed; the R.P.C.F.T. and the S.T.S. on his serum were still both positive. No T.P.I. tests were performed on sera from these last two patients.

(C) Problem Sera.—The sera in this group were mainly from patients whose S.T.S. had been found positive on routine testing, a T.P.I. test having been requested to check the specificity of these reactions. Sera from a small number of patients who presented lesions possibly attributable to syphilis, but whose S.T.S. were either negative or equivocal, have also been included. The results of the various tests are analysed with reference to the T.P.I. test in Table IV.

TABLE IV

COMPARISON OF RESULTS OF TESTS ON 144 PROBLEM SERA

Test	Result	Problem Sera	
		T.P.I. Reactive	T.P.I. Non-Reactive
F.T.A.	R	41	2
	N.R.	17	84
R.P.C.F.T. ..	R	24	8
	N.R.	34	78
S.T.S.	R	49	75
	N.R.	9	11
Total		58	86

These sera form a representative sample of those submitted to the laboratory for T.P.I. testing and it seems clear that the F.T.A. test showed a close agreement with the T.P.I., particularly where this test had been found negative, suggesting that the S.T.S. reactions might be non-specific. Reactive F.T.A. tests were found in only two instances where the T.P.I. test was negative. One patient was a woman with rheumatoid arthritis whose W.R. was weakly positive and R.P.C.F.T. negative; it is thought that this F.T.A. test was probably non-specific. The second patient was a woman whose F.T.A. and R.P.C.F.T. were both positive but whose S.T.S. and T.P.I. were negative. Her daughter had

been found to have positive S.T.S. and a congenital infection was queried. It would seem that the possibility that the positive results of tests on the mother's serum were due to syphilis cannot be excluded.

The proportion of non-reactive fluorescence tests in the T.P.I.-reactive group of sera was rather unexpected in view of the high sensitivity which the test had shown at all stages of treponemal infection (Tables I to III). It may be mentioned, however, that eight out of the seventeen sera classed as non-reactive did, in fact, show weak (+) fluorescence which was not sufficiently intense to be called reactive. The majority of the T.P.I. results obtained on these seventeen sera were either doubtful or only weakly positive. The eight sera giving (+) fluorescence produced 100 per cent. specific immobilization in two cases, 50 to 90 per cent. in five, and below 50 per cent. in one. The nine sera which were completely non-reactive in the F.T.A. test gave 50 to 90 per cent. specific immobilization in five cases, and below 50 per cent. in four. Among these seventeen patients, eight had clinical evidence suggestive of old syphilis and two were West Indians who gave a history of yaws in childhood.

Discussion

Theoretically the visual demonstration of the union of serum antibody with the specific antigen—*T. pallidum*—should afford a specific test for treponemal infection. The relationship between the antibody revealed by the F.T.A. test and the antibody or antibodies responsible for immobilization, agglutination and immune adherence of treponemes is not yet known. Preliminary experiments have suggested that it is possible to absorb out the reagin activity of a serum without diminishing the titre of the serum in the F.T.A. test, and that absorption with Reiter treponemes removed reactivity in the R.P.C.F.T. but had no effect on the F.T.A. titre of the serum. These findings, together with the negative F.T.A. results with sera giving non-specific reactions with tissue extract antigens as judged by negative T.P.I. results (Table IV *supra*), suggest that the antibody concerned in the F.T.A. differs both from reagin and from that responsible for the R.P.C.F.T. In this connexion the observation of Hardy and Nell (1957), that reagin antibody can agglutinate *T. pallidum* and hence presumably unite with it, presents a problem for future study. Their observation that fresh saline suspensions of *T. pallidum* were relatively or completely inagglutinable, but became agglutinable on storage in the refrigerator or on heating, suggested that reagin antibody may only react with a deeper-lying antigen in the treponeme which is exposed by these procedures. Deacon and Freeman (1960) state that fixation of rabbit anti-V.D.R.L. antigen globulins to treponeme suspensions prepared by the method of Hardy and Nell can be demonstrated by fluorescence procedures; they suggest that this may be due to the presence of normal testicular antigen deposited on the treponeme surface during the process of extraction.

The results obtained during the present preliminary study suggest that the presence of antibody, as shown by the fluorescence technique, closely parallels the presence of immobilizing antibody. Borel and Durel (1959) reported that the F.T.A. test titres were considerably higher than the titre of immobilizing antibody. The results of quantitative tests on a small number of sera bear this out (Table V).

TABLE V

COMPARISON OF TITRES GIVEN BY FIVE SERA

Clinical Category	Serum Titre			
	F.T.A.	T.P.I.	R.P.C.F.T	Cardiolipin W.R.
Untreated Secondary Syphilis	3,200	660	128	256
	1,600	100	256	64
Untreated Latent Syphilis	1,600	630	32	4
	6,400	540	128	256
Pooled Positive Serum	3,200	510	16	32

The F.T.A. test appears to have a high level of reactivity at all stages of untreated syphilis, and the findings in the small number of sera from patients with untreated primary lesions suggest that antibody is demonstrable by this method at an early stage of the infection. This agrees with the results of the S.E.R.A. Study in which the F.T.A. was found to be reactive in 60 per cent. of 130 cases of untreated primary syphilis in comparison with 25 to 50 per cent. of reactive T.P.I. tests using various techniques. More recently Montgomery, Suhrland, and Knox (1960) reported that 53 out of 58 sera from patients with untreated primary syphilis gave reactive F.T.A. tests, compared with 38 with the V.D.R.L. test and 27 with the R.P.C.F.T. Harris, Bossak, Deacon, and Bunch (1960) have shown that antibody was demonstrable by the F.T.A. test in the spinal fluid of 82 out of 369 patients with treated syphilis, while the T.P.I. test only gave 26 reactive or weakly reactive results on the same material.

The results of tests on the sera from the group of presumed non-syphilitic individuals suggest that the F.T.A. test has a high level of specificity. They compare favourably with the finding in the S.E.R.A. Study that the group of 346 presumed normal patients (American servicemen) gave only 1·5 per cent. reactive results.

The present investigation was designedly a preliminary study and therefore no firm conclusions are warranted. The test is technically easy to perform and, although much wider experience is needed—particularly of its reproducibility and susceptibility to quantitation, it appears to be a very promising addition to the group of tests based on the use of specific treponemal antigens.

Summary

(1) Fluorescent treponemal antibody tests have been performed on the sera of 167 presumed normal individuals, on 158 patients with treated or untreated treponemal disease (25 early, 92 latent, 41 late), and on 144 "problem" sera.

(2) The technique of the test is described. It is relatively simple to perform and the preliminary results obtained suggest that it has a high level of sensitivity and specificity.

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Test fluorescent des anticorps tréponème

Résumé

(1) L'auteur a pratiqué des tests fluorescents des anticorps tréponème sur le sérum de 167 personnes présumées normales, de 158 malades atteints de tréponématose traitée et non traitée (25 cas peu avancés, 92 cas latents et 41 cas avancés) et sur 144 prélèvements de sérum "problématique".

(2) La technique du test est décrite. Le test est relativement simple et les résultats préliminaires obtenus suggèrent qu'il possède un degré élevé de sensitivité et de spécificité.